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Treatment of Vivaspin® concentrators for improved recovery of low-concentrated protein samples

Application Note

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turning science into solutions

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Introduction

With appropriate device size and membrane cut-off selected, Vivaspin® products will typically yield recoveries for the concentrated sample > 90% when the starting sample contains over 0.1 mg/ml protein of interest. Depending on sample characteristics relative to the membrane type used, solute (protein) adsorption on the membrane surface is typically very low (2–10 µg/cm^2) and in practice not detectable.

This can increase to 20–100 µg/cm^2 when the filtrate is of interest and the sample must pass through the whole internal structure of the membrane. Whilst the relative adsorption to the plastic of the sample container will be proportionately less important than on the membrane, due to the higher total surface area, this can be also be a source of yield loss. Typically, a higher cut-off membrane will bind more than a low molecular weight alternative.

Whenever possible, the smallest MWCO and device size applicable should be chosen. Swinging bucket rotors are preferred to fixed angle rotors. This reduces the surface area of the concentrator that will be exposed to the solution during centrifugation.

An important factor not to be neglected is the thorough recovery of the retentate. Make sure to carefully remove all traces of solution from the sample container and, if feasible, rinse the device after recovering the sample with one or more drops of buffer and then recover again.

The intention of the following "passivation" procedure is to improve recovery of protein samples in the nano- to microgram concentration range by pretreating the device (membrane & plastic). For this purpose a range of solutions are suggested in Table 1.

Type	Concentration
Powdered milk	1% in Arium water
BSA	1% in PBS
Tween 20	5% in Arium water
SDS	5% in Arium water
Triton X-100	5% in Arium water
PEG 3000	5% in Arium water

Table 1: Passivation Solutions

Passivation procedure for Vivaspin® ultrafiltration concentrators

A) Passivation Procedure

1. Wash the concentrators once by filling with Arium water and spin the liquid through according to the respective protocol.

2. Remove residual water thoroughly by pipetting. **Caution: Take care not to damage the membrane with the pipette tip.**

3. Fill concentrators with the blocking solution of choice as given in Table 1.

4. Incubate the filled concentrators at room temperature for at least 2 hours (overnight is also possible except **for Triton X-100 which is not recommended for overnight incubation).**

5. Pour out the blocking solution.

6. Rinse the device 3–4x very thoroughly with Arium water and finally spin through.

7. The "passivated" devices are now ready for use. We recommend comparing different passivation reagents with an untreated device.

Note
It is necessary to rinse the device thoroughly before each washspin to ensure that traces of passivation compound are removed from the deadstop. Use the device immediately for protein concentration or store it at 4°C filled with Arium water, to prevent the membrane from drying.

B) Evaluation of passivation effects (exemplary with BSA)

1. Prepare a 10 µg/ml BSA stock solution e.g. by diluting 90 µl of the 4 mg/ml stock solution in 36 ml 0.1 M sodium borate pH 9.3. Mix well.

2. Fill Vivaspin® 2 devices with 2 ml of this 10 µg/ml BSA solution and close with cap provided.

3. Spin the device in a swing-out rotor at 4,000 × g until the volume is to app. 100 µl.

4. Recover the concentrate and make back up to 2 ml with 0.1 M sodium borate pH 9.3

5. Determine recovered protein concentrations e.g. according to Bradford or BCA assays.

Results and Discussion
As an example, the effect of milk powder was analysed. It could be shown (Table 2) that the protein recovery of a 10 µg/ml BSA solution could be increased from around 70 to 90%.
If milk powder is not interfering with sample purity and quality, it is a good starting point to improve recovery of diluted sample solutions.

Protein recovery (10 µg/ml BSA) with Vivaspin® PES 10 kDa after passivation
In another example, detergents were analysed with only 250 and 500 ng BSA (Table 3). BSA recovery declined to 50-30% in untreated devices as the protein concentration was reduced. Significant improvement to 60-90% recovery could be demonstrated when using the passivation strategy. Often, Triton X-100 seemed to work though the optimal reagent has to be selected for the respective protein and its hydrophilic|-phobic characteristics.

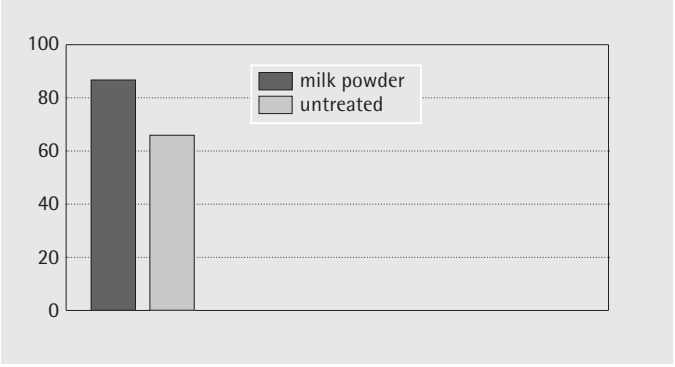

Table 2: Protein recovery (10 µg/ml BSA) with Vivaspin® PES 10 kDa after passivation

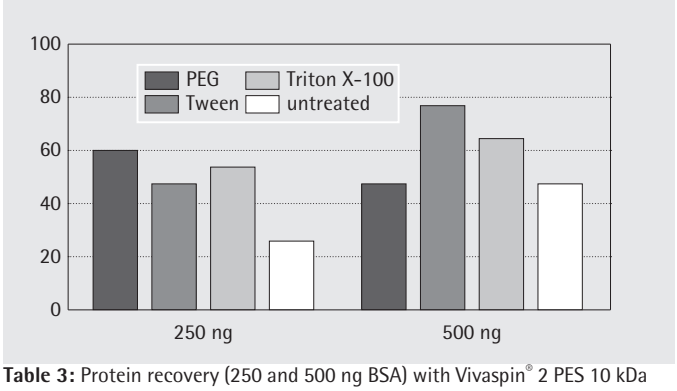

Table 3: Protein recovery (250 and 500 ng BSA) with Vivaspin® 2 PES 10 kDa after passivation

Summary
Passivation is an appropriate method to achieve increasing sample recovery when using very dilute samples. In addition to skimmed milk, other proteins (BSA), detergents and compounds are possible. However, it should be noted that this is a general procedure, not specific for any particular application. Depending on the hydrophilic|-phobic character of the protein non-specific binding may be more or less of a problem and the suggested passivation solutions may lead to different results. Even with the Hydrosart membrane, which is recommended for dilute samples, passivation of the device will reduce losses on the plastic surface. One very important thing to remember is that the blocking agent is potentially introduced into the sample. It should be assured that this will not interfere with downstream analysis. For example, proteins must not be used for passivation if a pure protein is intended to be concentrated for x-ray crystallography, as even the smallest traces would interfere with the diffraction pattern. Other subsequent analyses methods include activity testing, gel electrophoresis or labelling are less problematic.

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